IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Richard N. Zare		
Assignee:	The Board of Trustees of the Leland Stanford Junior University		
Title:	Bonded Phase Photopolymerized Sol-Gel Column and Associated Methods		
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DECLARATION UNDER 37 C.F.R. 1.132

We, Richard N. Zare, Maria T. Dulay, Joselito P. Quirino, and Bryson D. Bennett, declare the items set forth below.

1. We, Richard N. Zare, Maria T. Dulay, Joselito P. Quirino, and Bryson D. Bennett, are the inventors ("Inventors") named in the above-referenced United States Patent Application No. 09/978,515 ("Application").

2. Claims 1-11 ("Claims") of the Application, as filed, were rejected in an Office Action mailed on June 4, 2003 ("Office Action"). In the Office Action, the Claims were rejected under 35 U.S.C. Section 102(a), or alternatively, under 35 U.S.C. Section 103(a), on the basis of an article ("Article") by Maria T. Dulay, Joselito P. Quirino, Bryson D. Bennett, Masaru Kato, and Richard N. Zare ("Authors"), entitled *Photopolymerized Sol-Gel Monoliths for Capillary Electrochromatography*, that was published in Analytical Chemistry, Vol. 73, No. 16, August 15, 2001, 3921-3926.

3. We Inventors are co-authors of the above-referenced Article, along with our co-author, Masuru Kato ("Our Co-Author").

4. We Inventors invented certain subject matter ("Subject Matter") disclosed in the above-referenced Article that is the subject matter of the Claims.

5. The inventorship of the Application is correct in that it names us as the Inventors of the claimed invention, including the above-referenced Claims, and in that the above-referenced Article discloses Subject Matter that was derived from us, rather than invented by Our Co-Author of the Article.

6. Each of us declares that all statements made herein of my own knowledge are true, all statements made herein on information and belief are believed to be true, and all statements made herein are made with the knowledge that whoever, in any matter within the jurisdiction of the Patent and Trademark Office, knowingly and willfully falsifies, conceals, or covers up by any trick, scheme, or device a material fact, or makes any false, fictitious or fraudulent statements or representations, or makes or uses any false writing or document knowing the same to contain any false, fictitious or fraudulent statement or entry, shall be subject to the penalties including fine or imprisonment or both as set forth under 18 U.S.C. 1001, and that violations of this paragraph may jeopardize the validity of the application or this document, or the validity or enforceability of any patent therefrom.

August 11, 2003
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Anal. Chem. 2001, *73*, 3921−3926

Photopolymerized Sol−Gel Monoliths for Capillary Electrochromatography

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A solution of methacryloxypropyltrimethoxysilane in the presence of an acid catalyst, water, toluene, and a photoinitiator was irradiated at 365 nm for 5 min in a 75-μm i.d. capillary to prepare a porous monolithic sol−gel column by a one-step, in situ, process. The photopolymerized sol−gel (PSG) column shows reversed-phase behavior. Using this column, a variety of low-molecular-weight neutral compounds, including polycyclic aromatic hydrocarbons, alkyl benzenes, alkyl phenyl ketones, and steroids are separated from mixtures. Various different operational parameters, such as buffer composition, field strength, and column temperature, were varied to assess their influence on column performance. Use of PSG as a stationary phase for a pressure-driven separation is also demonstrated.

Capillary electrochromatography (CEC) has been regarded as a very promising analytical separation technique that combines the efficiency of capillary zone electrophoresis with the selectivity of liquid chromatography. Although CEC has been applied in many different areas,[1−6] packed-column preparation and low-detection sensitivity remain challenges of this technique. Capillary columns containing small silica packings have been the mainstay of CEC.[6,7] One disadvantage of packed columns is the fabrication of porous frits of controlled pore sizes, lengths, and high mechanical stabilities. In response to frit fabrication problems, surface-functionalized open-tubular capillary columns[6,8−10] and monolithic capillaries[6,8,11−14] are being developed as variants of packed capillary columns. Monolithic capillary columns have received much attention because of the advantages offered in the control of permeability and surface charge.

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We describe here the in situ one-step preparation and characterization of a photopolymerized sol−gel (PSG) having a porous structure and the ability of a PSG capillary column to separate neutral species in a liquid stream by application of an electric field. As a demonstration, a mixture of polycyclic aromatic hydrocarbons (PAHs) in a solution of aqueous acetonitrile has been pressure-injected onto the PSG column. This column has also been used to analyze for alkyl benzenes and alkyl phenyl ketones in mixtures.

EXPERIMENTAL SECTION

Materials and Chemicals. Fused-silica capillaries (75-μm i.d. × 365-μm o.d.) were purchased from Polymicro Technologies (Pheonix, AZ). Methacryloxypropyltrimethoxysilane (MPTMS) was purchased from Gelest (Tullytown, PA) and Sigma-Aldrich (Milkwaukee, WI) and was used without purification. HPLC-grade toluene, acetonitrile, tetrahydrofuran (THF), thiourea, naphthalene, phenanthrene, pyrene, alkyl benzenes, alkyl phenyl ketones, and steroids were purchased from Sigma-Aldrich (Milkwaukee, WI). Irgacure 1800 was received from Ciba (Tarrytown, NY).

Instrumentation. A Beckman P/ACE 2000 capillary electrophoresis instrument with a UV-absorbance detector was used to carry out all CEC experiments. An XL-1500 UV cross-linker (Spectronics Corp., Westbury, NY) equipped with six 15 W blacklight tubes of predominantly 365-nm wavelength was used to irradiate the reaction solutions. Scanning electron microscopy (SEM) analyses were performed on a Philips SEM 505 scanning electron microscope (Eindhoven, Netherlands).

Polymerization Procedure. The monomer stock solution was prepared just prior to use by adding 375 μL of MPTMS to 100 μL of 0.12 N HCl. This solution was stirred at room temperature for approximately 30 min to afford a clear, monophasic solution. A sol−gel colloidal precursor of the PSG is formed in this solution through a series of hydrolysis (1) and condensation (2,3) reactions. Hydrolysis can proceed to completion to form a fully hydrolyzed silane (1) or stop at a partially hydrolyzed silane, $Si(OR)_{4-n}(OH)_n$. The colloidal precursor may consist of dimers, trimers, and cyclic oligomers. At room temperature, gelation to form larger structures occurs at a very slow rate.

Hydrolysis: $$Si(OR)_4 + 4H_2O \rightarrow Si(OH) + 4ROH \quad (1)$$

Condensation:

$$2(RO)_3SiOH \rightarrow (RO)_3Si{-}O{-}Si(OR)_3 + H_2O \quad (2)$$

$$(RO)_4Si + (RO)_3SiOH \rightarrow (RO)_3Si{-}O{-}Si(OR)_3 + ROH \quad (3)$$

Photopolymers having morphologies with different permeabilities

Table 1. Amounts of Toluene Used for the Preparation of PSG Photopolymers

capillary column	% toluene (v/v)
A	90
B	80
C	75
D	73
E	65
F	50

and surface areas were prepared by adding the appropriate amount of toluene (porogen) to the monomer stock solution (see Table 1). The photoinitiator, Irgacure 1800, was added first to the toluene as 5% of the total weight of the toluene/monomer stock solution.[15] This photoinitiator solution was then added to the corresponding amount of monomer stock solution, and stirred for 30 min at room temperature to afford a yellow, monophasic solution. To minimize the evaporation of toluene, the solution was prepared in a vial with a polysilicone cap through which the capillary was inserted during filling with the solution.

A 15-cm stripe of the polyimide coating on a 30-cm long capillary was removed using a razor blade positioned at 45° to the capillary surface. The mechanical stability of the capillary is remarkably good despite the removal of a stripe of polyimide coating. The irradiation light entered the capillary only through this 15-cm stripe. No monolith was formed in the capillary where the polyimide coating ("mask") remained intact.

Using a 0.5-mL disposable syringe, approximately 0.2 mL of the reaction solution was flushed through the capillary to wet thoroughly the wall surface before filling the capillary with the solution. This resulted in bonding of the monolith to the capillary wall. No special pretreatment of the capillary wall was necessary to bond the monolith to the wall. The filled capillaries were irradiated (900 mJ/cm^2) in a UV cross-linker using 365-nm light for 5 min to form the PSG.

After irradiation, the capillaries were washed with ethanol using a hand-held syringe to remove unreacted reagents. Because the monoliths were highly permeable, high pressures were not required to drive liquid through the capillaries. Once the unreacted reagents were removed, the monolith became opaque and could be viewed clearly through the capillary without the aid of a microscope. The homogeneity of the PSG was confirmed at 100X magnification. Burning off the polyimide coating immediately after the monolith section with fuming sulfuric acid made a detection window.

Once fabricated, the capillary was successfully installed in the cartridge without any damage. The PSG capillary was conditioned with the separation buffer for approximately 5 min using a syringe and a hand-held vise. Once in the instrument, the capillary was further conditioned by pressure rinsing (20 psi) with the separation buffer or by electrokinetically conditioning at 5 kV or 10 kV for 30 min.

Characterization. SEM was used to study the morphology of PSG. A PSG capillary was sectioned carefully to expose the monolith. The sectioned pieces of capillary were sputtered with gold prior to SEM analyses.

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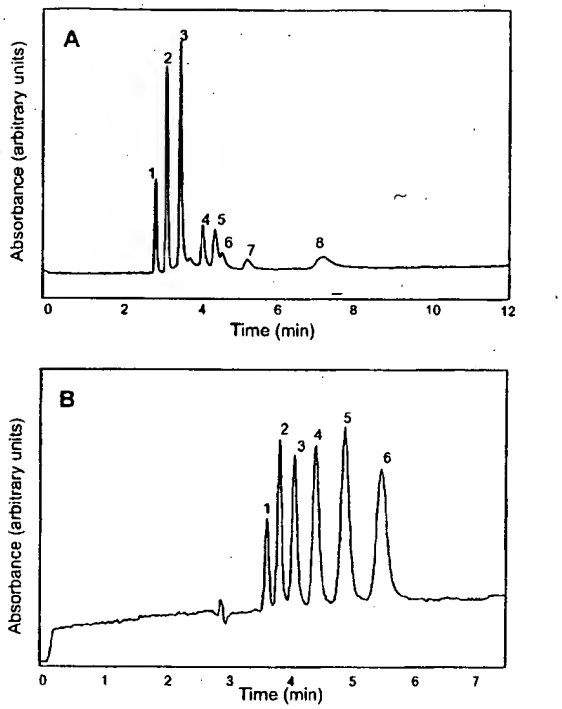

Figure 1. (A) Electrochromatogram of the separation of thiourea (1), tetrahydrofuran (2), and PAHs. The elution order of the PAHs is naphthalene (3), phenanthrene (4), fluoranthene (5), pyrene (6), 1,2-benzanthracene (7), and 1,2,5,6-dibenzanthracene (8). PSG column B, sample solution and mobile phase, 50 mM ammonium acetate/water/acetonitrile (1/3/6); 0.5 psi pressure injection, 3 s; applied voltage, 1 kV; temperature, 20 °C; detection, 214 nm. (B) Electrochromatogram of the separation of alkyl benzenes. The elution order is benzene (1), toluene (2), ethyl benzene (3), propyl benzene (4), butyl benzene (5), and hexyl benzene (6). PSG column D; sample solution and mobile phase, 50 mM ammonium acetate/water/acetonitrile (1/4/5); 0.5 psi pressure injection, 3 s; applied voltage, 15 kV; temperature, 20 °C; detection, 200 nm.

Analyte Separation. The analytes were prepared in the mobile phase to prevent gradient effects during the CEC experiments. The mobile phase was made up of various ratios (v/v) of 50 mM ammonium acetate, water, and acetonitrile. A new sample solution was used for every injection to maintain the same concentration of acetonitrile in the sample solution and the mobile phase.

RESULTS AND DISCUSSION

Construction and Characterization of PSG Columns. In addition to the short preparation time, the photochemical route to the preparation of PSG has many advantages: (1) control of the pore size, (2) control over the placement and length of the PSG segment, (3) high mechanical strength, and (4) avoidance of high temperatures that lead to cracking. The separation of thiourea, THF, and a series of PAHs is illustrated in Figure 1A using capillary column B (see Table 1), and the separation of a series of alkyl benzenes is illustrated in Figure 1B using capillary column D. Solute partitioning between the mobile and stationary phases is the only mechanism responsible for retention of the neutral analytes. The elution order of the column is similar to that of reversed-phase chromatography with the larger molecular weight or more hydrophobic analytes eluting later than the smaller molecular weight or more hydrophilic analytes. Elution of

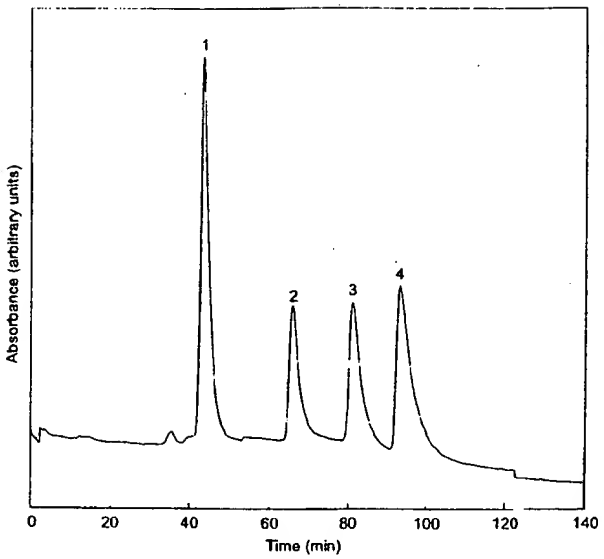

Figure 2. Chromatogram of the separation of thiourea (1), naphthalene (2), phenanthrene (3), and pyrene (4); PSG capillary D; sample solution and mobile phase, 50 mM ammonium acetate/water/acetonitrile (1/3/6); 0.5 psi injection, 3 s; separation pressure, 20 psi; temperature, 20 °C; detection, 214 nm.

the analytes in both figures occurs in less than 7 min. Bubble formation was not a problem during the CEC experiments, for which the typical operating currents were between 3 and 10 μA.

For a typical capillary column D (Table 1), efficiencies of up to 100 000 plates/m are achieved for thiourea, a less-retained compound.[16] Small variations in the elution times were observed for thiourea (0.65% RSD), naphthalene (1.10% RSD), phenanthrene (1.14% RSD), and pyrene (1.14% RSD) over a period of 3 days (n = 33).

The versatility of the PSG column for a pressure-driven separation of the test mixture is shown in Figure 2. A mixture of thiourea, naphthalene, phenanthrene, and pyrene is separated within 110 min at an applied pressure of only 20 psi (the maximum limit of our instrument). Peak tailing is most severe for pyrene because of its strong interaction with the PSG surface, and tailing is not observed for thiourea, which has low retention on the column.

Characterization of PSG formed with 80% (v/v) toluene (column B, Table 1) by SEM reveals an interconnecting network of 1-μm spherical structures through which micrometer-sized macropores (as large as 5 μm) are interspersed (see Figure 3A). These macropores serve as "through pores" that allow for mass transfer of the analytes from the mobile phase to the chromatographic active sites for separation. SEM analyses (not shown) also reveal that PSG is bonded to the capillary wall.

The permeability of PSG is determined by the linear velocity of the monolith, which is proportional to permeablity as described in Darcy's law.[17] The permeability of PSG as a function of the macropore size is highly dependent on the volume and type of porogen used to prepare the photopolymer. A porogen serves a

(16) An experiment in which NaBr and thiourea were injected into a PSG column showed that thiourea has some adsorption onto the column. Thiourea eluted slightly after the bromide peak.

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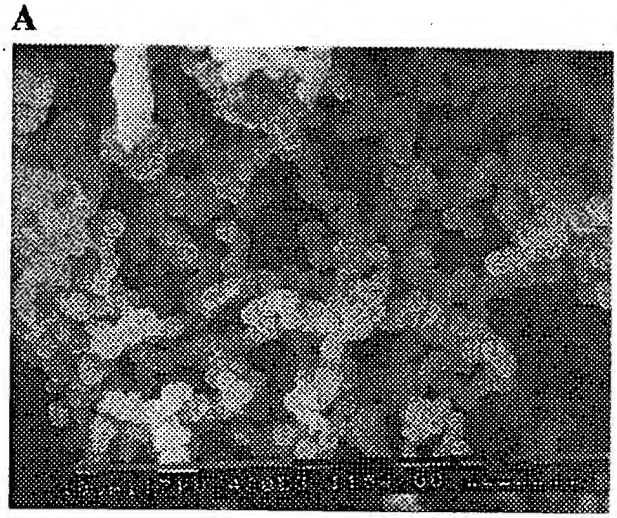

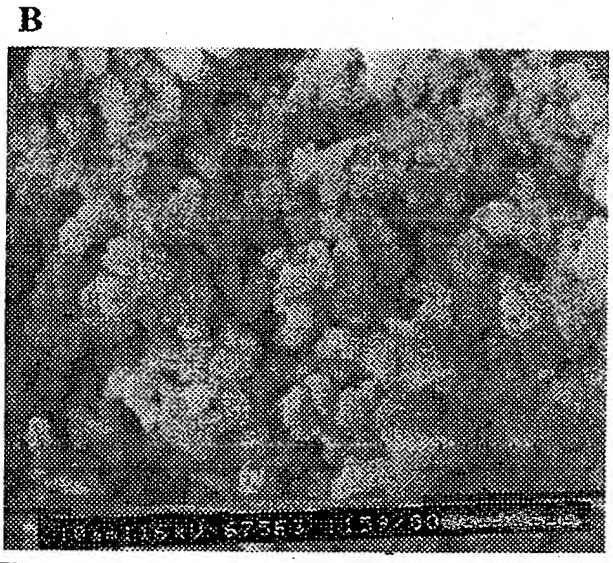

Figure 3. Scanning electron micrographs of (A) the cross-section of a PSG photopolymer formed with 80% (v/v) toluene (capillary B) in a 75-μm-i.d. capillary column and (B) the cross-section of a PSG photopolymer formed with 50% (v/v) toluene in a 75-μm-i.d. capillary column.

dual role as a pore template and a solubilizer. Toluene was found to be the best porogen in our experiments. For a column made with 90% (v/v) toluene (column A), the linear velocity is 12.3 cm/min, and an 80% (v/v) column (column B) has a linear velocity of 3.3 cm/min, whereas a column made with 73% (v/v) toluene (column D) has a linear velocity of 0.6 cm/min. When the amount of toluene is decreased to 50% (v/v), a dense PSG structure is obtained (Figure 3B). In contrast to the PSG shown in Figure 3A, this dense photopolymer has macropores of 2 μm or less in diameter. Consequently, this PSG is less permeable, and a significant back pressure occurs. No liquid could be driven through the column at pressures near 200 psi. The effect of higher pressures on liquid flow through the column was not assessed. These linear velocity data suggest that the macropores decrease with decreasing porogen concentrations. This behavior is consistent with what is reported in the literature.[18]

A variety of other porogens were used to prepare PSG. Acetonitrile, dimethylformamide (DMF), butanol, cyclohexane, cyclohexanol, acetone, iso-octane, THF, and different ratios of hexane/toluene and butanol/toluene were used as porogens. A photopolymerized sol–gel was not formed when cyclohexane, cyclohexanol, or isooctane was used as a porogen. This behavior may be a result of the poor solubilizing properties of these solvents. On the other hand, DMF, butanol, butanol/toluene, THF, and acetone solubilized the reagents, but PSG was not formed. A nonporous PSG (i.e., fluid cannot be flushed through at 200 psi) is formed when acetonitrile (a good solubilizer) is used as a porogen. A mixture of 1:1 hexane/toluene, however, afforded a permeable monolith with separation performance similar to that of a PSG made with 80/20 toluene/reaction solution. A column efficiency of 68 000 plates/m (RSD 7.0%, $n = 5$) for thiourea and an electroosmotic flow (EOF) velocity of 3.7 cm/min was obtained.

To achieve a wider range of selectivities and enhanced chromatographic performance, other chemical units were incorporated into the photopolymerized sol–gel by copolymerization of MPTMS with other monomers, including *o*-(methacryloxyethyl)-*N*-(triethoxysilylpropyl)urethane, bis(triethoxysilyl)nonane, tetramethoxysilane, and tridecyl methacrylate. But this procedure afforded monolithic structures with low separation performances and weak mechanical strengths.

The effect of sol–gel photopolymer length in the capillary on analyte elution times was studied for lengths of 15, 10, 5, and 0 cm from the window to the inlet. As the length of the segment decreases, the resolution decreases. As a result, the analytes coelute. Baseline separation of the test mixture of thiourea, naphthalene, phenanthrene, and pyrene was not achieved for lengths shorter than 10 cm. The generated currents, however, remained almost unchanged for the lengths of photopolymer we examined. The data indicate that only the mobile phase, and not the PSG, influences the conductance. In a packed column, the packed and open segments exhibit different conductances, and hence, different electric field strengths are produced in the column.[6] This behavior leads to a mismatch of the local electroosmotic flow velocities between the packed and open sections, which causes a decrease in column efficiency.[6,19] The electric field strengths in a PSG column, however, are homogeneous throughout the capillary. It follows that the electroosmotic flow velocity is constant through the column, so that broadening effects from velocity variations are not expected to occur.

At high field strengths, it is expected that peak broadening will occur as a result of Joule heating. Nevertheless, we observe that the peaks are sharpened further at higher field strengths. It appears that efficient dissipation of the Joule heat generated at high field strengths (667 and 1333 V/cm) occurs with the PSG. A plot of current versus applied voltage is linear ($R = 0.9998$) over the range of 5 to 25 kV, which suggests that there is little heating effect in the PSG capillary column.

Effect of Temperature. Temperature is a controllable parameter that can be used to further optimize CEC separations.[20,21] Figure 4 illustrates that when the column temperature was

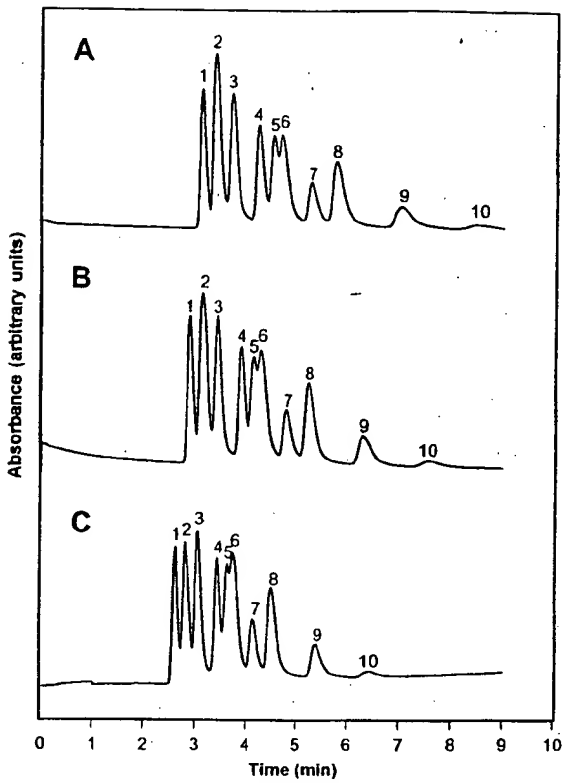

Figure 4. Electrochromatograms of the separation of PAHs at column temperatures of (A) 20, (B) 40, and (C) 50 °C; thiourea (1), tetrahydrofuran (2), naphthalene (3), phenanthrene (4), fluorene (5), pyrene (6), benz[*a*]anthracene (7), benz[*e*]acephenanthylene (8), benzopyrene (9), and coronene (10); PSG column D; sample solution and mobile phase, 50 mM ammonium acetate/water/acetonitrile (1/3/6); 0.5 psi pressure injection, 3 s; applied voltage, 10 kV; temperature, 20 °C; detection, 214 nm.

increased to 50 °C (in column D), the elution times of the analytes decreased as the electroosmotic flow velocity increased. This change in the electroosmotic flow velocity in the PSG column is ascribed only to changes in the mobile-phase viscosity and not to structural changes in the PSG structure as a function of temperature. Structural changes of isopropylacrylamide grafted to silica with temperature changes have been reported for other systems.[22]

The same trend of increasing elution time with decreasing temperature is observed for pressure-driven (20 psi) separations. This fact is not surprising, because temperature also has a strong influence on analyte retention in pressure-driven chromatography.[23] Table 2 shows the effect of temperature on the retention factors (k), where k is the ratio of the number of moles of solute in the stationary PSG matrix to that in the mobile phase. As the column temperature is increased, the retention factor for each analyte decreases slightly. The exception is naphthalene at 12 and 20 °C, where the k values are the same within experimental error.

Effect of Buffer Concentration. Changing the concentration of the ammonium acetate buffer in the mobile phase between 1

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Table 2. Retention Factor (k) as a Function of Temperature in Pressure-Driven Separations of PAHs[a]

temp, °C	k		
	naphthalene	phenanthrene	pyrene
12	0.36	0.64	0.86
20	0.37	0.67	0.92
30	0.34	0.60	0.82
40	0.29	0.53	0.72
50	0.28	0.51	0.68

[a] PSG capillary B; sample solution and separation solution, 50 mM ammonium acetate/water/acetonitrile (1/3/6); 0.5 psi pressure injection, 3 s; separation pressure, 20 psi; temp, 20 °C; detection, 214 nm.

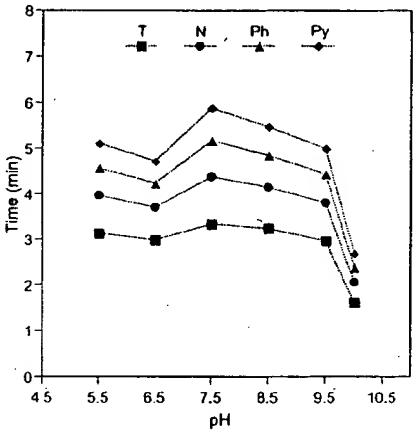

Figure 5. Effect of buffer pH on the elution times of thiourea (T), naphthalene (N), phenanthrene (Ph), and pyrene (Py). Conditions are the same as in Figure 5.

and 15 mM varied the ionic strength of the mobile phase.[6] We made a plot (not shown) of the buffer concentration versus the elution times of the analytes in our test mixture. The elution times increased as the ionic strength of the buffer in the mobile phase increased. A linear relationship is established between ionic strength and elution times in the buffer concentration range studied. The buffer concentration effect that is observed is expected for CEC separations.

Effect of Buffer pH. Figure 5 shows that the useful pH range is 5.5 to 10 for the separation of our test mixture. At this working pH range, electroosmotic flow velocities are stable. The total surface charge is probably unchanged at this pH range. Elution times drop dramatically at pH 10. It is known that dissolution of silica-based materials occurs at pH > 9.[24] At pH 11, the EOF was unstable. Similarly, at pH 4.5 (not shown), the EOF of the column was unstable, resulting in irreproducibility of elution times at the same experimental conditions. The instability of the column at low pH may be because of hydrolysis of the ester moiety in the PSG material.

Effect of Acetonitrile Concentration. Figure 6 illustrates the dependence of the elution times of a series of alkyl phenyl ketones on acetonitrile concentration in the mobile phase for column D. The acetonitrile composition of the mobile phase is in the range of 40 to 80% (v/v). Above 50% (v/v) acetonitrile, Figure 6 shows that the analytes are coeluted, with no separation attained at 80%

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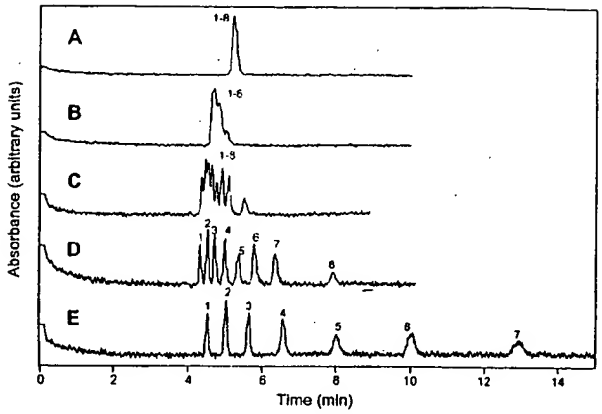

Figure 6. Electrochromatograms of the separation of alkyl phenyl ketones with acetonitrile concentrations of (A) 80, (B) 70, (C) 60, (D) 50, and (E) 40% in the mobile phase. The analytes are 1.42 μM acetophenone (1), 25 μM propiophenone (2), 15 μM butyrophenone (3), 20 μM valerophenone (4), 5.5 μM hexanophenone (5), 16.5 μM heptanophenone (6), 15.3 μM octanophenone (7), and 3.59 mM decanophenone (8). Conditions are similar to those in Figure 4.

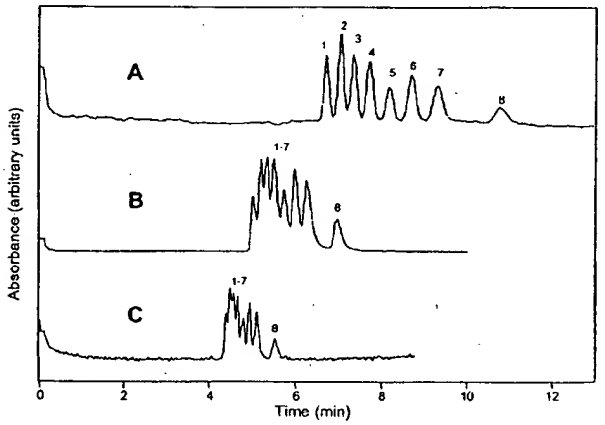

Figure 7. Electrochromatograms of the separation of alkyl phenyl ketones in (A) capillary A, (B) capillary B, and (C) capillary D (see Table 1). The analytes are 4.2 μM acetophenone (1), 75 μM propiophenone (2), μM butyrophenone (3), 60 μM valerophenone (4), 16 μM hexanophenone (5), 49 μM heptanophenone (6), 46 μM octanophenone (7), and 11 mM decanophenone (8). Conditions are the same as those in Figure 4.

(v/v) (Figure 6A). Some separation of the analyte peaks is observed for acetonitrile concentrations of 70 (Figure 6B) and 60% (v/v) (Figure 6C). At 50% (v/v) acetonitrile, baseline separation of all eight ketones is achieved in 8 min, with the peak shapes being symmetrical (Figure 6D). Improved baseline resolution is achieved using an acetonitrile concentration of 40% (v/v), as shown in Figure 6E. Partitioning of the analytes from the mobile phase to the monolith phase, which is hydrophobic, is affected by the amount of acetonitrile present in the mobile phase. At low volumes of acetonitrile (i.e., higher volumes of water), partitioning is altered in favor of the analytes; therefore, the analytes interact longer with the PSG surface. The changes in the elution times of the analytes with acetonitrile concentration are expected for a reversed-phase mechanism. In typical reversed-phase chromatography, the elution times decrease with an increase in organic solvent concentration in the mobile phase. For acetonitrile concentrations between 40

Table 3. Retention Factors (k) for 3 PAHs in Different Column Morphologies[a]

		ave k	RSD, %
90/10 (n = 4)	naphthalene	0.17	10
	phenanthrene	0.34	8.2
	pyrene	0.52	8.8
80/20 (n = 3)	naphthalene	0.39	0.0
	phenanthrene	0.69	0.86
	pyrene	0.96	0.62
73/27 (n = 4)	naphthalene	0.53	1.8
	phenanthrene	0.97	1.5
	pyrene	1.35	1.9

[a] Sample solution and separation solution, 50 mM ammonium acetate/water/acetonitrile (1/3/6); 0.5 psi pressure injection, 3 s; applied voltage, 10 kV; temp, 20 °C; detection, 214 nm.

and 60% (v/v), the elution times do not appear to change significantly.

Effect of the PSG Morphology. The separation abilities (for a sample of alkyl phenyl ketones) of three columns containing different sol–gel photopolymers are illustrated in Figure 7. In all three columns the analytes are eluted in order of increasing alkyl chain length, with acetophenone eluting first and decanophenone last. Baseline separation of the phenones in less than 12 min is achieved when the column has low permeability and high photopolymer content (Figure 7A, column D). With a decrease in the porogen concentration, there is a concomitant increase in the volume of monomer in the reaction solution; therefore, more of the photopolymer is formed. The resulting photopolymer affords a higher retention of the analytes on the sol–gel photopolymer. With a lower concentration of the monomer in the reaction solution, less photopolymer is formed in a column with which the analytes can interact. As a result, there is a lower retention of the analytes on the PSG surface, and separation of the analytes is poor, as shown in Figure 7B (column B) and C (column A) for the same experimental conditions. This behavior is illustrated in Table 3 for the separation of naphthalene, phenanthrene, and pyrene in the same columns. As the volume of the PSG increases, the k values increase, which is indicative of stronger retention of the analytes. Clearly, the ability to control the morphology of the PSG is important to successful separation of the sample mixture.

CONCLUSIONS

We have developed a simple and fast procedure for the in situ photopolymerization of a sol–gel monolith in a capillary column. The resulting photopolymer is well-suited for the separation of a variety of neutral compounds in the reversed-phase mode in both voltage-driven and pressure-driven systems.

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On Aug 11, 2003 (Date), before me, Anca Ruhlen, Notary Public (Name and Title of Officer (e.g., "Jane Doe, Notary Public")),
personally appeared Richard N. Zare, Maria T. Dulay, Joselito P. Quirino and Bryson D. Bennett (Name(s) of Signer(s))

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Notary Public - California
Santa Clara County
My Comm. Expires Aug 18, 2005

WITNESS my hand and official seal.

Anca Ruhlen
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